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Comparison of Antibody Responses to Different Forms of HIV-1 Core Antigens by Epitope Mapping

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The specificity of antibodies to HIV-1 capsid (p24CA) and matrix (p17MA) proteins, produced in mice against unprocessed immature assembled polyprotein (wild-type p55 virus-like particles or chimeric p55 virus-like particles) or against the monomeric mature form (rp24CA/rp17MA), was analyzed by a microplate epitope mapping assay using a panel of synthetic peptides covering the entire p24CA plus p17MA sequences of HIV-1_{LAI}. All immunized mice developed anti-p24CA and anti-p17MA antibodies, although the spectrum of specificity of these antibodies was different. Four p24 CA epitopes (residues 176–192, 201–218, 233–253, 285–304) were recognized by anti-rp24CA/rp17MA antibodies, whereas one p17MA epitope (residues 11–25) and one p24CA epitope (residues 176–192) were constantly recognized by anti-p55 virus-like particle antibodies. These results suggest a different specificity pattern of anti-p24CA and anti-p17MA antibodies depending on whether they are produced against the soluble mature form or the immature assembled form of the gag proteins. ***J. Med. Virol. 51:145–151, 1997.***

KEY WORDS: HIV-1; core precursor; virion assembly; virus-like particles; epitopes

INTRODUCTION

The gag gene of the human immunodeficiency virus type 1 (HIV-1) encodes a structural precursor polyprotein, p55, whose proteolytic processing yields four mature proteins, the capsid protein (p24CA), the matrix protein (p17MA), the nucleoprotein (p9NC), and the protein p6 [Veronese et al., 1988; Henderson et al., 1992]. Both p24CA and p17MA are considered as some of the most immunogenic HIV-1 antigens [Janvier et al., 1993] since an early and intense antibody response to p24CA and an early but lower intensity antibody response to p17MA are usually observed during natural exposure to HIV-1.

Major immunogenic domains of mature p24CA and p17MA have been characterized with murine monoclonal antibodies (MuMoABs) using synthetic peptides overlapping the entire capsid protein or matrix protein sequences. Several p24 B-cell epitopes were thus mapped within sequences 179–197, 203–217, 233–262, 273–292, 285–304, and 303–317 [Niedrig et al., 1989; Mathiesen et al., 1989; Wahren et al., 1989; Carpio et al., 1991; Janvier et al., 1996], whereas human sera bound only epitopes 179–197, 253–277, and one highly conserved epitope 285–304, also called the major homology region (MHR) [Matsuo et al., 1992; Janvier et al., 1996]. An immunodominant p17MA B-cell epitope was mapped within the C-terminal sequence 113–122, using MuMoAbs [Niedrig et al., 1989], while human sera bound epitope 113–122 as well as the sequence 12–36 located at the N-terminal part of p17MA [Mathiesen et al., 1989; Wahren et al., 1989].

The p24CA B-cell epitopes are presented differently from the immune system depending on whether soluble protein or intact virions are used as immunogens [Mabrouk et al., 1992]. We developed a microplate epitope mapping assay to characterize the specificity of the core antibody response induced by either monomeric mature forms (rp24CA, rp17MA) or unprocessed immature assembled polyprotein (wild-type [WT] or chimeric p55 virus-like particles [VLPs]). We report a differential specificity pattern of anti-p24CA as well as anti-p17MA antibodies depending on the immunogen presentation.

MATERIALS AND METHODS

Immunogens

Recombinant soluble p24CA (rp24) and p17MA (rp17) were produced in *Escherichia coli* transfected with the gag gene sequences coding, respectively, for p24CA and p17MA of HIV-1/HXB2 [Cheynet et al., 1993].

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Recombinant particulate antigens were produced in *Spodoptera frugiperda* insect cells (SF9) infected with either a WT gag (WT p55) or a chimeric gag-env recombinant baculovirus. WT p55 is assumed to involve all the necessary structural features required for direct assembly into the capsid shell as well as into VLPs. We described previously a strategy for construction of chimeric gag-env genes using a polymerase chain reaction (PCR) procedure, which allowed us (*i*) to generate gag-env recombinant baculoviruses by cotransfection of recombinant gag-env baculoviral transfer vector with *Autographa californica* nuclear polyhedrosis viral DNA, (*ii*) to perform the expression of the recombinant proteins in SF9 cells for an optimal VLP formation, and (*iii*) to purify the recombinant VLPs from the supernatant of infected SF9 cells [Brand et al., 1995; Truong et al., 1996]. According to this procedure, a WT recombinant gag gene and two chimeric gag-env genes were generated expressing the principal neutralization determinant of the V3 loop (p55V3) [Javaherian et al., 1990; Larosa et al., 1990] or a linear epitope located within the CD4 binding site (p55CD4BS) [Lasky et al., 1987] (Fig. 1A). As reported previously, the deletion-insertion site within the p24CA (residues 196–228) corresponded to an immunologically relevant epitope. Details for construction, expression, purification, and analytical controls have been described elsewhere [Truong et al., 1996]. The three constructs yielded VLPs of 100–120 nm in diameter, similar to the immature core particles observed during replication of HIV-1 (Fig. 1B).

Immunization Protocols

WT p55-, p55V3-, and p55CD4BS-purified particles were used to immunize 8-week-old female Balb/c mice. A mixture of purified rp24CA and rp17MA was used as control immunogen. Three mice were used for each preparation. Every mouse was immunized on days 0, 21, 42, and 72 with 25 μg of immunogen. Immunization on day 0 included complete Freund's adjuvant and subsequent injections incomplete Freund's adjuvant. Blood samples were collected before immunization (P0) and 1 week after the last boost (P2).

Characterization of the Whole gag Antibody Response

The gag antibody response was examined in sera from all immunized mice by enzyme-linked immunosorbent assay (ELISA) using the entire p24CA or p17MA. One hundred microliters per well of rp17MA (0.28 μg/ml) or rp24CA (0.1 μg/ml) in 0.05 M bicarbonate buffer, pH 9.6, was used to coat microtiter plates (Microtest III flexible assay plate; Becton Dickinson, Oxnard, CA) by incubation overnight at 37°C. Plates were then washed three times with phosphate-buffered saline (PBS buffer), 0.01 M, pH 7.4, 0.15 M NaCl containing 0.5% Tween 20, and quenched by incubation for 45 min at 37°C with 200 μl of PBS containing 5% nonfat dry milk (Régilait, Lyon, France). After three washes, 100 μl of 1:100 and 1:1,000 dilutions of test sera in PBS containing 2% nonfat dry milk and 0.5% Tween 20 was added and incubated for 1 hr at 37°C. Wells were washed five times, and 100 μl of horseradish peroxidase-conjugated goat anti-mouse F(ab′)2 (Tago, Burlingame, CA), diluted 1:5,000 in test sera buffer, was added and incubated for 1 hr at 37°C. Plates were then washed five times, and 100 μl of a mixture containing hydrogen-peroxide-o-phenylenediamine was added at room temperature in the dark. Color development was stopped 30 min later with 50 μl of 2 N H_2SO_4 and absorbance (as optical density [OD]) read at 492 nm. The cut-off for each assay was determined by using the mean OD value obtained with the preimmune sera (PO) from 12 mice plus 3 standard deviations (SD).

Characterization of the Specificity of the gag Antibody Response

The epitope-specific gag antibody response of murine sera was examined by ELISA using a panel of synthetic peptides covering p24CA and p17MA sequences.

Thirty-three overlapping synthetic peptides, homologous to the sequence covering amino acids 1–132 of p17MA (SP1 to SP13) and 133–363 of p24CA protein (SP14 to SP33) of the HIV-1_{LAI} strain, were kindly provided by the Agence Nationale de Recherche sur le SIDA (ANRS, Paris, France). Peptides were 15 mer long on average (extremes 12–21). Peptide sequences are given in Figure 2.

Each peptide (1 μg/ml) in 0.05 M bicarbonate buffer, pH 9.6, was used to coat a different well of the microtiter assay plate (Microtest III flexible assay plate; Becton Dickinson) by incubation overnight at 37°C. Murine sera were tested at 1:100. The reaction procedure was carried out as described above for rpl7MA and rp24CA assays. A peptide was defined as containing an epitope when bound by at least two of three sera from mice immunized with a given antigen, with a minimum absorbance equal to 0.2.

Antibody Response to Envelope Inserts

The antibody response of murine sera toward HIV-1 envelope inserts was examined by ELISAs using microtiter plates coated with either 0.1 μg of consensus B V3 peptide for anti-V3 antibodies or 0.05 μg recombinant surface envelope glycoprotein rgp120SU for anti-CD4BS antibodies. The V3 antibody assay was an indirect ELISA already described [Truong et al., 1996]. The CD4BS antibody assay was a competition assay using a labeled human MoAb [Turbica et al., 1995].

RESULTS

Antibody Responses to rp24CA and rp17MA

Antibody responses to both p24CA and p17MA were observed in the sera of all immunized animals. Mature forms rp24CA/rp17MA and the immature form WT p55

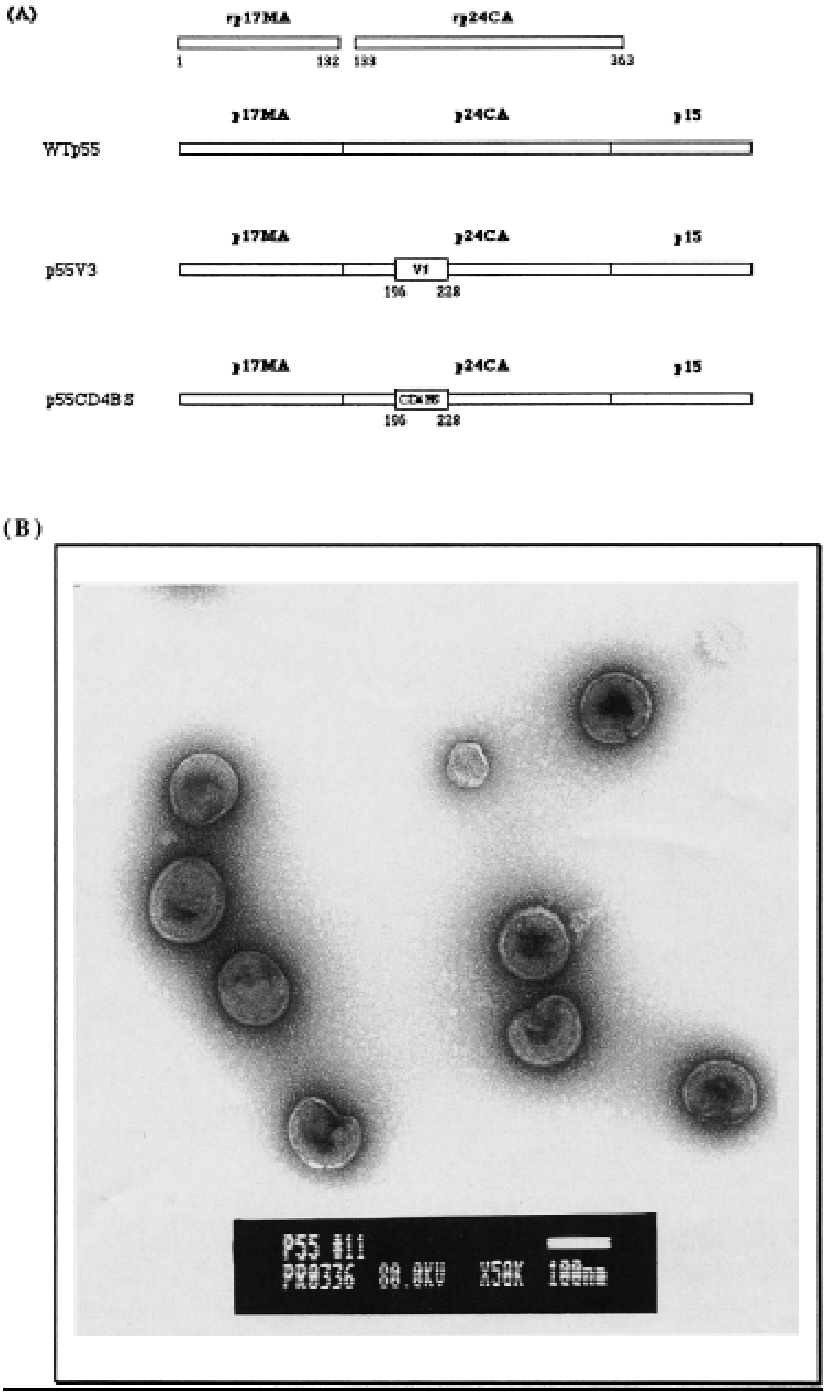

Fig. 1. Description of the immunogens. **A:** Schematic representation of the various constructs. **B:** Electron micrograph showing recombinant WT p55 particles.

induced a high antibody response in mice (Table I). However, the binding capacity of these sera to p24CA was at least 10-fold higher than their binding capacity to p17MA, as shown by respective dilution sera. This ratio also characterized antibody response during the natural course of infection [Janvier et al., 1993]. The binding capacity of these sera to either p24CA or p17MA was relatively similar regardless of the immunogen, except that the matrix response appeared slightly lower in mice immunized with the WT p55 VLPs when compared to those immunized with the recombinant mature form. In contrast, chimeric VLPs p55V3 and p55CD4BS induced a lower response to both p24CA and p17MA (Table I).

SP1	15-mer : sequence 1-15	M G A R A S V L S G G E L D R
SP2	15-mer : sequence 11-25	G E L D R W E K I R L R P G G
SP3	15-mer : sequence 21-35	L R P G G K K K Y K L K H I V
SP4	16-mer : sequence 31-46	L K H I V W A S R E L E R F A V
SP5	15-mer : sequence 41-55	L E R F A V N P G L L E T S E
SP6	15-mer : sequence 51-65	L E T S E G C R Q I L G Q L Q
SP7	17-mer : sequence 62-78	G Q L Q P S L Q T G S E E L R S L
SP8	17-mer : sequence 70-85	T G E E L R S L Y N T V A T L Y
SP9	16-mer : sequence 81-96	T V A T L Y C V H Q R I E I K D
SP10	15-mer : sequence 93-107	E I K D T K E A L D K I E E E
SP11	12-mer : sequence 104-115	I E E E Q N K S K K K A
SP12	13-mer : sequence 111-123	S K K K A Q Q A A A D T G
SP13	12-mer : sequence 121-132	D T G H S N Q V S Q N Y
SP14	16-mer : sequence 133-148	P I V Q N I Q G Q M V H Q A I S
SP15	15-mer : sequence 142-156	M V H Q A I S P R T L N A W V
SP16	19-mer : sequence 154-172	A W V K V V E E K A F S P E V I P M F
SP17	16-mer : sequence 169-184	I P M F S A L S E G A T P Q D L
SP18	17-mer : sequence 176-192	S E G A T P Q D L N T M L N T V G
SP19	16-mer : sequence 193-208	G H Q A A M Q M L K E T I N E E
SP20	18-mer : sequence 201-218	L K E T I N E E A A E W D R V H P V
SP21	15-mer : sequence 219-223	H A G P I A P G Q M R E P R G
SP22	21-mer : sequence 233-253	G S D I A G T T S T L Q E Q I G W M T N L
SP23	17-mer : sequence 245-261	E Q I G W M T L N P P I P V G E I
SP24	15-mer : sequence 256-270	I P V G E I Y K R W I I L G L
SP25	15-mer: sequence 263-277	K R W I I V G L N K I V R M Y
SP26	18-mer : sequence 270-287	L N K I V R M Y S P T S I L D I R Q
SP27	20-mer : sequence 285-304	I R Q G P K E P F R D Y V D R F Y K T L
SP28	19-mer : sequence 302-320	K T L R A E Q A S Q E V K N W M T E T
SP29	19-mer : sequence 319-337	E T L L V Q N A N P D C K T I L K A L
SP30	17-mer : sequence 335-350	K A L G P A A T L E E M M T A C Q
SP31	15-mer : sequence 346-360	M M T A C Q G V G G P G H K A
SP32	16-mer : sequence 357-372	G H K A R V L A E A M S Q V T N
SP33	15-mer : sequence 364-378	A E A N S Q V T N S A T I M M

Fig. 2. Amino acid sequences of the synthetic peptides (SP) derived from p24CA and p17MA HIV-1_{LAI}. Synthetic peptides recognized by each category of murine sera are depicted by boxes on the right. Black boxes correspond to peptides recognized by sera from rp24CA/rp17MA-immunized mice. White boxes correspond to peptides recognized by sera from p55-immunized mice. Hatched boxes correspond to peptides recognized by sera from p55V3- or p55CD4BS-immunized mice.

Epitope Mapping

Mapping of the B-cell epitopes was carried out by ELISA using a panel of 12–21 residue synthetic peptides (Fig. 2).

The mixture of soluble mature rp24CA and rp17MA induced antibodies which recognized four linear epitopes located exclusively within p24CA (Fig. 3A). Anti-rp24CA/rp17MA murine sera bound epitopes within SP18 (residues 176–192), SP20 (residues 201–218), SP22 (residues 233–253), and SP27, which contains the MHR (residues 285–304). Binding activity was the highest for SP22 and SP27. Surprisingly, no linear p17MA epitope was recognized by anti-rp17MA sera despite their rp17 reactivity.

Immature particulate antigens induced antibodies which recognized linear epitopes within p24CA as well as within p17MA. Anti-WT p55 sera bound three p24CA epitopes within SP18, SP22, and SP27 and, interestingly, one p17MA epitope within SP2 (residues 11–25) (Fig. 3B). Chimeric p55V3 and p55CD4BS VLPs induced only antibodies to SP18 within p24CA (respectively, Fig. 3C, D). Both induced antibody to SP2 within p17MA, confirming that this region is probably more immunogenic when exposed in the immature polyprotein than in the soluble mature form. Two other regions of p17MA (51–65 and 81–96) appeared immunogenic but only in the p55CD4BS construct (Fig. 3D).

Only chimeric p55V3 VLPs induced a significant but,

TABLE I. Antibody Responses to p24CA (Sera Diluted 1:1,000) and p17MA (Sera Diluted 1:100) of Individual Animals Immunized With the Various Core Antigens

	Mature form	Virus-like particles		
	rp24CA + rp17MA	WTp55	p55V3	p55CD4BS
Anti-p24CA 1:1,000				
Mouse 1	⩾3.5	⩾3.5	⩾3.5	2.413
Mouse 2	⩾3.5	⩾3.5	0.897	0.165
Mouse 3	⩾3.5	2.689	0.223	0.121
Mean OD ± SD	⩾3.5	3.23 ± 0.46	1.54 ± 1.73	0.9 ± 1.310
Cut-off value = 0.026				
Anti-p17MA 1:100				
Mouse 1	1.538	1.189	0.414	0.321
Mouse 2	0.946	0.933	0.443	0.379
Mouse 3	1.887	0.457	0.124	0.014
Mean OD ± SD	1.46 ± 0.48	0.86 ± 0.37	0.327 ± 0.176	0.238 ± 0.196
Cut-off value = 0.017				

Values correspond to absorbance (OD) at 492 nm. The cut-off for each assay was determined by using the mean OD values obtained with the preimmune sera (P0) from 12 mice plus 3 standard deviations (SD).

nevertheless, low anti-V3 response (data not shown). CD4BS antibody response was not detected with our assay regardless of the immunogen.

DISCUSSION

The humoral immune response to HIV-1 gag proteins p24CA and p17MA was analyzed in mice using an epitope mapping assay in order to study the importance of the antigenic presentation context. Therefore, mice were immunized with equivalent amounts of either mature recombinant rp24CA and rp17MA proteins or immature WT p55 or chimeric p55 assembled into VLPs.

Particulate or aggregated antigens, which are claimed frequently to be good immunogens, do not appear in the current study to be more potent immunogens than the soluble monomeric forms of recombinant proteins. Indeed, immature WT p55 assembled into VLPs did not appear more immunogenic than the soluble forms of mature proteins since antibodies present in immunized animals did not show any major differences in binding capacities as assayed by ELISA. Moreover, the deletion of a short p24CA sequence followed by the insertion of a heterologous epitope clearly lowered the core antibody response (Table I). Alteration of the antibody response to p24CA in animals immunized with the chimeric VLPs could be explained at least in part by the deletion of a p24CA epitope. However, both p55V3 and p55CD4BS VLPs also induced a lower antibody response to p17MA, although no deletion was introduced in this region. This result clearly indicates that modification of the p24CA sequence may alter the antibody response not only to p24CA itself but also to other regions of the polyprotein p55, possibly via conformational changes.

Differential specificity patterns of core antibody responses elicited by the various immunogens were characterized using an epitope mapping assay (Fig. 3). Although soluble monomeric form rp17MA induced antibodies able to bind the whole p17MA in an ELISA format, we did not identify any linear p17MA epitope by peptide mapping, suggesting that the majority of these antibodies were directed to conformational epitopes. Surprisingly, either WT or chimeric VLPs induced antibodies reacting to the same linear p17MA epitope located within residues 11–25 (Fig. 3B–D). Therefore, we characterized a sequence specifically immunogenic in the immature assembled polyprotein p55. This sequence has been shown previously to be immunogenic during natural infection since it is bound by sera from HIV-1-infected individuals [Wahren et al., 1989]. Based on recent crystallographic study, this region, which is positively charged, might be exposed in a loop presented at the outer periphery of the p17MA trimers that constitute the network of the matrix shell [Matthews et al., 1994; Rao et al., 1995]. It was also shown to be exposed on the surface of immature WT p55 assembled into VLPs using immunoelectron microscopy [Carrière et al., 1995].

The soluble form of rp24CA induced antibodies reacting to four linear sequences (Fig. 3A) identified as B-cell epitopes in several previous studies [Niedrig et al., 1989; Mathiesen et al., 1989; Wahren et al., 1989; Janvier et al., 1990; Carpio et al., 1991; Matsuo et al., 1992; Janvier et al., 1996]. Only one of these sequences was still immunogenic in any of the three different VLPs (Fig. 3B–D). It corresponds to residues 176–192, a sequence also bound by sera from infected humans [Mathiesen et al., 1989; Janvier et al., 1996], recombinant p55-immunized chimpanzees [Mabrouk et al., 1992], and recombinant p24CA-immunized rabbits [Mabrouk et al., 1992] and mice [Janvier et al., 1990]. Therefore, we characterized an immunogenic sequence exposed in the mature p24CA as well as in the immature assembled polyprotein p55. WT p55 VLPs induced antibodies to three of these four p24CA epitopes. Indeed, the sequence 201–218 was not immunogenic when presented in the immature assembled form (Fig. 3B). Therefore, we characterized a sequence specifically immunogenic in the mature p24CA. The chimeric VLPs were able to induce only antibodies reacting to sequence 176–192 (Fig. 3C, D). In particular, the MHR

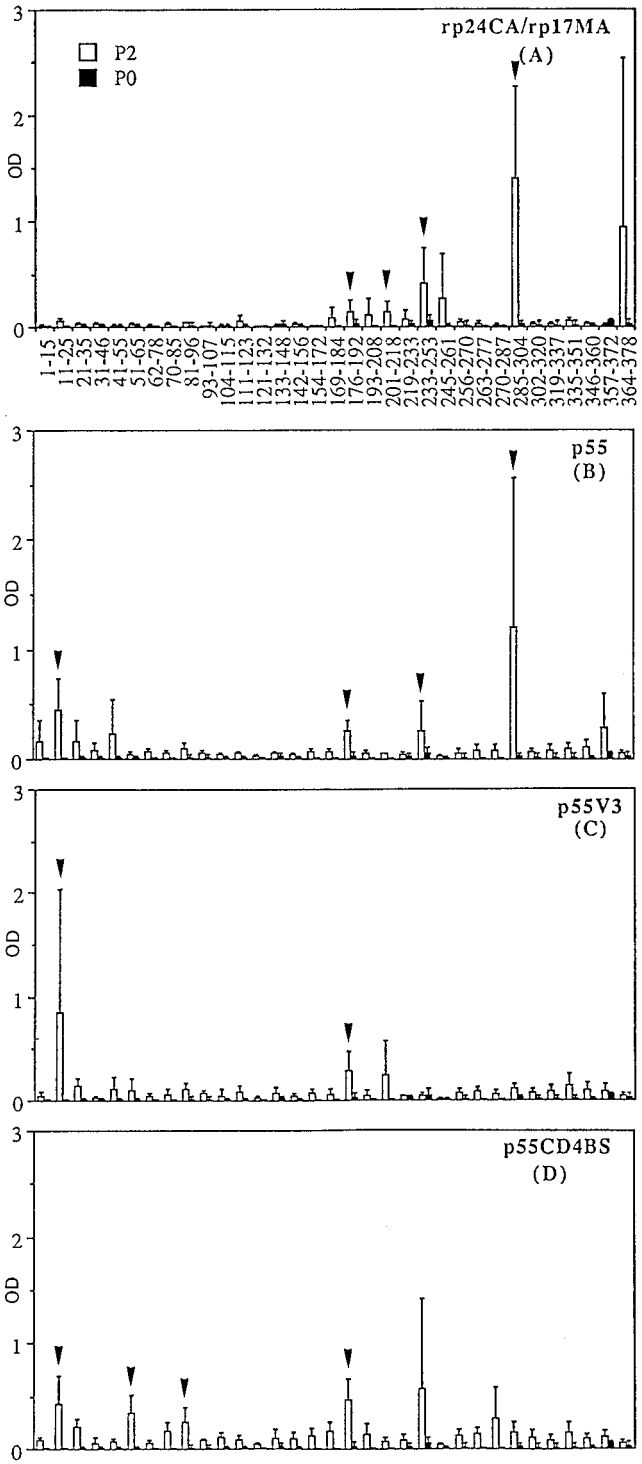

Fig. 3. Characterization of the specificity of the gag antibody response for sera from mice immunized with rp24CA/rp17MA **(A),** WTp55 **(B),** p55V3 **(C),** and p55CD4BS **(D).** Each serum was assessed at 1:100 with all 33 synthetic peptides. Each histogram represents an average result between three sera from mice immunized with the same antigen. Samples P0 and P2 correspond to preimmune and immune sera, respectively. Standard deviation is also represented for each bar. Arrows indicate synthetic peptides which contained an epitope defined as peptide bound by at least two of three sera from mice immunized with the same antigen, with a minimal OD of 0.2.

(residues 285–304), which was highly immunogenic in the WT protein in our study and is highly immunogenic during natural infection [Carpio et al., 1991; Janvier et al.,1 996], did not induce antibodies when presented in the chimeric constructs. In contrast, the p55CD4BS VLPs induced antibodies able to bind two additional epitopes within the p17MA region. Taken together, these data clearly show that the insertion of heterologous epitopes in core-like particles modified and determined their immunogenicity. Such an observation was also made and a similar conclusion reached when neutralizing epitopes of the pre-S region of the hepatitis B virus envelope were inserted at different places of the viral core protein [Schödel et al., 1992].

The capsid of various viruses has been suggested to be a carrier moiety for vaccine purposes [Moriarty et al., 1990]. Our results indicate and support the idea that the nature and the position of the inserted epitope is critical for immunogenicity and that manipulation of the specificity of the humoral response must be studied extensively before consideration as optimal immunogens.

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